2015. The Journal of Arachnology 43:97–100

A re-evaluation of the formula to estimate the volume of orb web glue droplets

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Abstract. The size and shape of the glue droplets along the spiral threads of orb webs play an important role in web function. Despite this, methods for estimating droplet volume are not well defined, with contradicting formulas published. Here we address the discrepancies in the published formulas with a mathematical derivation that assumes that a glue droplet conforms to a parabola along one side of the axial line. We confirmed the validity of our derived formula by comparing it with the results of numerical integration. We also document that a droplet continues to conform to a parabola as its volume changes with environmental humidity. Our formula can be applied simply by collecting the spiral threads, examining the droplets under a light microscope and measuring their length and width, making it easy to compare the droplets of different species collected at different relative humidities.

Keywords: Aggregate glue, computer simulation, mathematical proof, spiral threads

The volume, shape, number and chemical composition of glue droplets on orb web spiral threads have important consequences for the adhesive properties of orb webs (Opell 2002; Opell & Hendricks 2007; Opell et al. 2011b, 2013; Sahni et al. 2011, 2014; Torres et al. 2013). As such, many recent studies have estimated glue droplet volume to ascertain how orb web spirals might function across a range of scales (e.g., Opell & Hendricks 2007, 2009, 2010, Opell & Schwend 2007, 2009; Wu et al. 2013; Blamires et al. 2014).

The glue of orb webs is spun fully coating the underlying pair of flagelliform fibers. The compounds in the glue render it hygroscopic and, as a consequence, the glue readily absorbs atmospheric moisture (Edmonds & Vollrath 1992; Higgins et al. 2001; Sahni et al. 2011; Stellwagen et al. 2014). A combination of internal forces and surface tension creates Rayleigh instability so the glue forms into droplets (Vollrath & Edmonds 1989, 2013; Edmonds & Vollrath, 1992; Opell et al. 2013); the same forces that make water in air form into droplets. Under a light microscope, the droplets appear parabolic and periodically positioned along the flagelliform fibers resembling "beads along a string" (Sahni et al. 2012; Torres et al. 2013; Wu et al. 2013).

Estimating the volume of the glue droplets is generally done by collecting samples of spiral silk from webs, examining them under a light microscope at 100×–1000× magnification, and measuring the length (l) of the droplet along the capture thread's paired axial lines and the width (w) of the droplet perpendicular to the axial lines. The volume, V, of an individual orb web spiral droplet has, in most cases, been estimated from the formula:

$$V=\frac{2\pi(w)^2 l}{15} \tag{1}$$

(Opell & Schwend 2007, 2009; Agnarsson & Blackledge 2009; Opell & Hendricks 2009, 2010; Wu et al. 2013; Stellwagen et al. 2014). This formula is a corrected version of a formula first used to determine the percentage of material invested in smaller secondary droplets of viscous threads (Opell & Hendricks 2007) and was reported as:

$$V=\frac{2\pi(h)^2 b}{15} \tag{2}$$

where the values h and b appear to have been incorrectly defined as "h = 0.5 droplet width and b = 0.5 droplet length". This paper therefore: (i) formally addresses the discrepancy in the formulas used to compute droplet volume, (ii) provides the first detailed mathematical derivation of the corrected formula, and (iii) confirms the validity of the corrected formula using a computer simulation.

Furthermore, the compounds in the glue from orb-web viscous threads confers hygroscopicity, thus droplet volume changes with environmental humidity (Townley et al. 2006; Opell et al. 2011a; Opell et al. 2013; Townley & Tillinghast 2013). We thus confirmed the applicability of our formula for droplet volume computation under different conditions by determining whether droplet shape remained a parabola over a wide range of humidities.

FORMULA REEXAMINATION

We derived a droplet volume formula based on the conclusion that the profile of a droplet on one side of the axial line assumed the configuration of a parabola and that droplet volume can, therefore, be computed as the volume encompassed by a parabola rotated around this axis. The requirement that a droplet's profile be a parabola was confirmed for droplets of *Leucauge venusta* (Walckenaer 1841), *Metepeira labyrinthea* (Hentz 1847), *Araneus pegnia* (Walckenaer 1841), and *Araneus marmoreus* Clerck 1757. For these species the droplets' regressions of coordinates predicted by a parabola against those measured on droplet images had R^2 > 0.97 (Opell & Hendricks 2007). Therefore, each quadrant of a droplet represents a parabola (Fig. 1) whose coordinates are expressed as:

$$y=h(1-(x/b)^2),\ 0\le x\le b \tag{3}$$

where $b = l/2$ and $h = w/2$ (Opell & Hendricks 2007).

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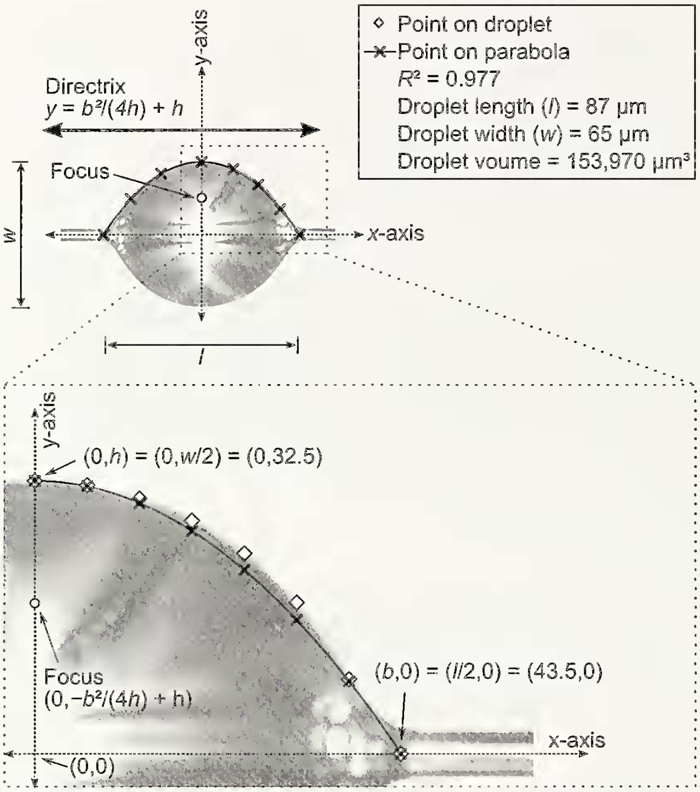

Figure 1.—Viscous thread droplet of *Araneus marmoreus* photographed at 90% relative humidity, showing fit of coordinates to a parabola, reference points used in volume formula computation, and the volume computed for this droplet.

Accordingly, droplet volume is calculated by rotating y around the x-axis and multiplying this value by 2, so

$$V=\frac{16\pi h^2 b}{15} \tag{4}$$

Substituting $w = 2h$ and $l = 2b$, we obtain formula (1), which allows the measured dimensions of droplets to be used directly in computing droplet volume. Details of the derivation of equation (4) follow.

A parabola is defined as the points equidistant between a line, called the directrix, and a point, called the focus. To derive the equation for the parabola that approximates the outline of a droplet on the axial line pair of an orb-weaving spider's web, we placed the axial lines on the x-axis and the widest part of the droplet on the y-axis. We assigned the length of the droplet along the x-axis as l and the width of the droplet along the y-axis as w; see Fig. 1. We let $b = l/2$ and $h = w/2$. The right-hand endpoint of the droplet on the x-axis is $(b, 0)$. The top point of the droplet on the y-axis is $(0, h)$, and we call the distance between this point and the directrix and between this point and the focus $p/2$.

The focus is the point $(0, -((p/2) - h))$, which equals $(0, (h - (p/2)))$; the equation for the distance to the directrix at point $(b, 0)$ is

$$y=h+(p/2) \tag{5}$$

and the distance to the focus is

$$y=sqrt\,(b^2+(h-(p/2))^2)=sqrt\,(b^2+(p^2/4)-p\,h+h^2). \tag{6}$$

Since the distances (5) and (6) are equal in a parabola, we equate these equations, square both sides, and simplify:

$$\begin{aligned}(h+(p/2))^2&=b^2+(p^2/4)-p\,h+h^2\\ p/2&=b^2/4h\end{aligned} \tag{7}$$

The distance between point (x,y) on the parabola and the directrix is its distance to point $(x, (p/2)+h)$ on the directrix:

$$sqrt\,(y-((p/2)+h))^2. \tag{8}$$

The distance between point (x,y) on the parabola and the focus point $(0, (h - (p/2)))$ is

$$sqrt\,(x^2+(y-h+(p/2)^2)). \tag{9}$$

If we substitute equation (7) for p/2 and then equate equations (8) and (9), then squaring both sides and simplifying, we obtain for the equation of the curve:

$$y=h\,(1-(x^2/b^2)) \tag{10}$$

The volume, V, of the droplet is

$$V=2\,\pi\int_0^b y^2\,dx. \tag{11}$$

$$\begin{aligned}V&=2\,\pi\,\textstyle\int_0^b(h(1-(x^2/b^2)))^2\,dx\\ &=2\,\pi\,h^2\,\textstyle\int_0^b(1-(x^2/b^2))^2\,dx\\ &=2\,\pi\,h^2\,\textstyle\int_0^b\,(1-2\,(x/b)^2+(x/b)^4)\,dx\\ &=2\,\pi\,h^2\,(x-(2\,x^3/(3\,b^2))+(x^5/(5\,b^4)))]_0^b\end{aligned}$$

$$V=\frac{16\pi h^2 b}{15} \tag{4}$$

Substituting $b = l/2$ and $h = w/2$ to express the formula in terms of directly measured droplet dimensions, we obtain:

$$V=\frac{2\pi(w)^2 l}{15} \tag{1}$$

To check whether equation (2) or equation (4) provides the closest estimate of the volume of a parabola, we ran a numerical integration to simulate 1,000,000 parabola subsections added together using the program R (R Development

Table 1.—Mean dimensions and volumes of viscous thread droplets from five adult female *Argiope aurantia* at three humidities (± standard deviation).

Relative humidity (%)	Droplet length (μm)	Droplet width (μm)	Droplet width/length	Droplet volume (μm^3)
17.8 ± 2.2	63.6 ± 5.5	45.0 ± 5.5	0.74 ± 0.04	54,950 ± 14,856
55.0 ± 0.7	67.2 ± 5.5	48.4 ± 5.3	0.71 ± 0.05	67,337 ± 19,002
90.8 ± 0.8	82.2 ± 7.5	61.0 ± 5.8	0.72 ± 0.05	130,381 ± 32,806

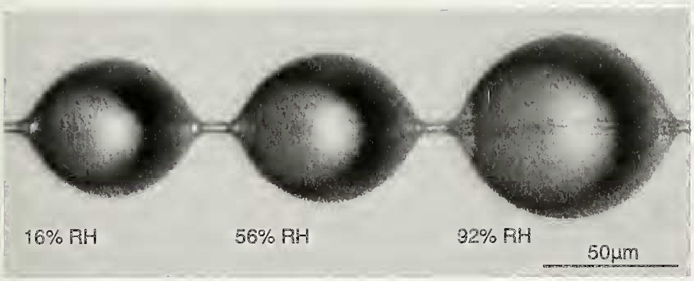

Figure 2.—Photograph of the same droplet of an individual *Argiope aurantia* at three humidities.

Core Team 2010). We used a quadrature rule based on interpolating functions with 1,000,000 permutations. Assigning an h value of 2 and b value of 5 we found an answer of ~ 67.020, which is what equation (4) finds, with equation (2) finding a much underestimated answer of ~ 8.378.

When we re-ran the simulation using an h value of 4 and b value of 10, we found an answer of ~536.135, which is consistent with what equation (4) finds. When l = 2 and w = 5, however, our answer of ~31.41 more closely approximates what equation (2) finds; confirming that there was an error in defining the variables h and b in formula (2).

APPLICATION

Does droplet shape change with humidity?—We examined the droplets from the viscous threads spun by five adult female *Argiope aurantia* Lucas 1833, whose threads were collected near Blacksburg, Montgomery Co., Virginia during September 2010. Using techniques described previously (Opell et al. 2011a), we transferred threads from web sectors to microscope slides and recorded the positions of individual droplets by identifying the position of a thread strand on the sampler and the position of the droplet by noting its sequential position from a support. We photographed each droplet at the same magnification at 17, 55, and 90% relative humidity, within a temperature range of 23–24 °C and measured droplet length and width with an OndeSoft® screen protractor (Beijing Torrentsoft Technology Co., Ltd. Beijing, China) using an image of a stage micrometer as a scale.

After inserting each of these 15 droplet images into a PowerPoint® (version 14.46 for Mac 2011, Microsoft Inc., Mountain View, CA) slide, we drew lines through the droplets' horizontal and vertical axes, and positioned 12 small points along the outline of the upper right quarter of the droplet such that they were equally spaced on the X axis that bisected the droplet (Fig. 1). We then recorded the coordinates of each point and used the two outermost points to construct a parabola. From each droplet's X coordinate, we computed its Y coordinate as predicted by the parabola and compared these predicted coordinates to the measured coordinates to determine if a droplet's shape matched that of a parabola. The outermost points on each droplet were excluded from the analysis, as these were used to compute the parabola contour against which the other ten points were compared.

At each humidity, droplet contour assumed the shape of a parabola (Fig. 2). The ratio of droplet width to length (Table 1, Fig. 3), which was normally distributed (Shapiro-Wilk test $P > 0.11$), did not differ among humidities (ANOVA P = 0.5156). Thus we confirmed that the formula described in this study is appropriate for across-humidity comparisons of droplet volumes.

CONCLUSION

The volume of a viscous thread droplet of an orb web can be reliably estimated as that of a parabola rotated around its axis by equation (4). Because the forces driving the formation of droplets are the same among orb web spiders, the formula can be universally applied, facilitating more meaningful comparisons between studies. It will be easier and less complicated, in practice, if the formula is expressed in terms of the droplet length and width dimensions l and w rather than the parabola coordinates h and b. By showing that a viscous droplet continues to assume the shape of a parabola over a wide range of humidities, we demonstrated that the formula has broad applicability for measuring the volumes of all spiral droplets. This test also shows how an investigator can confirm this fit in other situations.

ACKNOWLEDGMENTS

Brent Opell acknowledges support from a National Science Foundation grant (1257719). Sean Blamires acknowledges support from an Australian Research Council grant (DE140101281).

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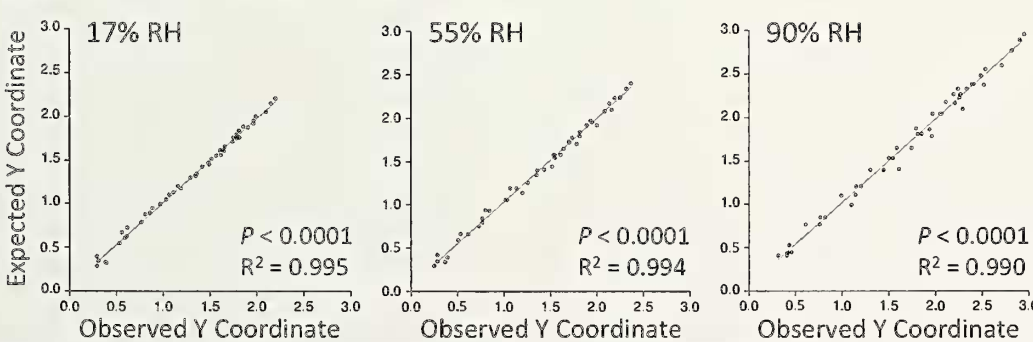

Figure 3.—Evaluation of the fit of *Argiope aurantia* viscous droplets to a parabola at three humidities. Each regression plots the expected and observed Y coordinates of ten points along the contour of each of the five individual's droplets.

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Manuscript received 2 September 2014, revised December 17 2014.